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Effects of tank-mixing of micronutrients and amitraz by foliar application on abundance of *Agonoscena pistaciae* in pistachio orchards

Mohammad ROUHANI*, **Mohammad Amin SAMIH**
Department of Plant Protection, Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran

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Abstract: Fertilization is one of the effective factors on the common pistachio psylla *Agonoscena pistaciae* Burckhardt and Lauterer (Hem.: Aphalaridae) population. Fertilization has a central role in all metabolic processes, as well as in the cellular structure, cell wall structure, and growth of all organisms, and various pesticides are now used to control this pest, as well. The aim of this study was to determine the effects of a pesticide (amitraz) mixed with micronutrients (Ca, Zn, and N) on the population density of *A. pistaciae*. We tested the effects of mixing pesticides and micronutrients on *A. pistaciae* in a pistachio orchard. The effect of nutritional solutions on nymph and egg populations showed a significant difference at a 5% concentration level. The highest measure of control of the eggs was related to combination of amitraz + N + Zn and amitraz + N + Ca, while the least effective was amitraz + Ca. The results also showed that the highest measure of nymph control was related to the combination of amitraz + N+ Zn+ Ca, while the least was related to amitraz alone and amitraz + Zn.

Key words: *Agonoscena pistaciae*, amitraz, nutrition element, pesticide

1. Introduction

The pistachio, *Pistacia vera* L. (Anacardiaceae), is one of the most important horticultural products in Iran, Armenia, Iraq, Turkmenistan, and Turkey and is known as the green-gold tree (Ozden-Tokatli et al. 2005; Alizadeh et al. 2007). The common pistachio psylla, *Agonoscena pistaciae* Burckhardt and Lauterer (Hem.: Aphalaridae), is one of the most significant pests of pistachio trees due to its widespread distribution in all pistachio-producing regions of Iran. Both nymphs and adults suck the sap of leaves and produce large amounts of honeydew. Direct feeding reduces plant vigor and encourages defoliation, stunting, poor yield, and bud drop (Samih et al. 2005). Nowadays various pesticides are used to control the common pistachio psylla. The control of this population has been based on pesticides with no attention paid to the pest density and possible economic damage. The economic injury level is an important component of a cost-benefit analysis in integrated pest management (IPM) programs and is a useful tool for decision making in the application of pesticides (Shipp et al. 2000; Hassani et al. 2009). Part of the defensive mechanisms of plants against pathogens or herbivorous insects is related to the synthesis of secondary plant metabolites or allelochemicals (Fragoyiannis et al. 2001). Researchers have suggested the increasing pressure on agroecosystems from insect pests and diseases is due to changes that have occurred in agricultural practices since World War II. The use of fertilizers and pesticides has increased rapidly during this period. Evidence suggests that such excessive use of agrochemicals in conjunction with expanding monocultures has exacerbated pest problems (Conway and Pretty 1991; Altieri and Nicholls 2003). Nitrogen is one of the most critical chemical elements for plant and animal growth (Daugherty et al. 2007). Calcium is an important element for cell wall structure and various physiological processes in trees (Littke and Zabowski 2007). Zinc is essential for normal healthy growth and reproduction of plants, animals, and humans (Sarwar 2011). The application of nitrogen as a fertilizer can normally increase the succulence in stems and leaves, which can lead to greater pests attack (Lu et al. 2007), and the application of calcium can decrease density of pests such as *Myzus persicae* (Luna 1988).

Tank-mixing of pesticides is a routine procedure that can reduce the cost of application, enhance the activity of certain products, and widen the range of treatments in a single application. When pesticides are combined with other poisons, fertilizers, and additives, it can cause chemical interactions, such as synergic, additive, and antagonist interactions (Petroff 2003). Therefore,

* Correspondence: rouhani_valiasr@yahoo.com

mixing must be approached carefully so that there are no antagonisms interfering with the efficacy of various components, synergisms that could cause crop damage, or chemical reactions creating unsprayable sludge (Gooding et al. 1991; Minbashi et al. 2006). Chemical compatibility does not guarantee that the mixture will perform as expected. If we want to tank-mix pesticides to increase the effectiveness of the application, there are some things to consider before we proceed. It is important to know that it is possible to mix pesticides with other products as nutrient elements (Sander et al. 1987; Bagherani 2005).

In this experiment, we investigated the effects of combining pesticides and nutrient elements on the population abundance of *A. pistaciae* and compared it with the use of pesticides in pistachio orchards of Rafsanjan, Iran.

2. Materials and methods

2.1. Study site

This study was conducted in pistachio orchards in Rafsanjan, Iran, during 2010 and 2011. Pistachios are the predominant tree variety in the study area. For this purpose, pistachio orchards with trees from Ahmadaghaei rootstock with similar ages and horticultural operations were selected. This test was applied on the basis of complete randomized blocks with 3 replications and 9 treatments, including amitraz + Zn, amitraz + Ca, amitraz + N, amitraz + Zn+ Ca, amitraz + N+ Ca, amitraz + N+ Zn, amitraz + N+ Zn+ Ca, amitraz alone, and the control (distilled water), applied to the common pistachio psylla fed on 20-year-old pistachio trees from Ahmadaghaei rootstock in the first year. According to the results of the first year, the following fertilizer treatments were selected for the experiment in the second year: amitraz + Ca, amitraz + Zn+ Ca, amitraz + N+ Zn, amitraz + N+ Zn+ Ca, amitraz alone, and the control. Two trees for each treatment were randomly assigned (18 treatments per block). The dates and concentrations were chosen based on the usual time recommended for spraying pistachio trees and the results of other research in the study area. Thus, concentrations of 0.5%, 0.3%, 0.05%, and 1 L/L were selected for nitrogen, calcium, zinc, and amitraz, respectively. Each of the required elements was obtained from Merck or Bayer (Germany). The spraying technique was used.

2.2. Sampling

Sampling began 72 h after spraying (middle of May 2010 and 2011) and continued at 4-day intervals until harvest (late August). For this purpose, each sample was separated. Five leaves from each side (20 leaves total) of each treated tree were taken and transferred to the laboratory. Data were recorded for the number of eggs and nymphs on top of and under each leaf. Based on 32 sampling dates and 20 leaves per treatment, the average number of each developmental stage was calculated per leaf.

2.3. Statistical analysis

The analysis of data was performed using SPSS 16 and the comparison of means by Duncan's test. The diagrams were designed using Excel software.

3. Results

3.1. First year

The results of the analysis of variance showed that the nutritional solution had an effect on the 1st instar nymphs ($F_{8,261}$ = 2.59, P = 0.01), 5th instar nymphs ($F_{8,261}$ = 4.84, P = 0.00), and the total amount of instar nymphs ($F_{8,261}$ = 2.80, P = 0.00) at a 1% concentration level and eggs ($F_{8,261}$ = 1.78 , P = 0.05), 2nd instar nymphs ($F_{8,261}$ = 1.95, P = 0.05), and 4th instar nymphs ($F_{8,261}$ = 1.92, P = 0.05) at a 5% concentration level. There was not a significant difference for 3rd instar nymphs ($F_{8,261}$ = 0.91, P = 0.56). Using Duncan's test, an analysis of the grouping and the similarity degree of the pest population treated with a 5% concentration level was carried out (Table 1).

As shown, the combination with the highest measure of influence on eggs was amitraz + N + Zn and the least amount of influence was found with amitraz + Ca, compared to the control (Figure 1). An increase of population of the 1st instar nymphs was caused by amitraz + Zn and amitraz alone, and there was a significant difference between treatments and the control. The results also showed that amitraz + N + Zn + Ca caused a decrease in the abundance of 1st instar nymphs, and there was no significant difference between the treatments and the control. Amitraz alone increased the abundance of 2nd instar nymphs and amitraz + Zn+ Ca decreased the density, but this difference was not significant. For the 3rd instar nymphs, there was no significant difference between the treatments and the control. Amitraz + Ca and amitraz alone also increased density of the 4th and 5th instar nymphs (Table 1). The study of abundance of nymphs in this test showed that amitraz increased their abundance significantly compared to the control, and amitraz + N + Zn + Ca and amitraz + NZn decreased the abundance of nymphs (Figure 2).

3.2. Second year

The results of the analysis of variance showed that the effect of the nutritional solution at a 1% concentration level had a significant difference on eggs ($F_{5,474}$ = 3.79 , P = 0.002), 1st instar nymphs ($F_{5,474}$ = 547.12, P = 0.00), 2nd instar nymphs ($F_{5,474}$ = 204.29, P = 0.00), 3rd instar nymphs ($F_{5,474}$ = 82.49, P = 0.00), 4th instar nymphs ($F_{5,474}$ = 89.59, P = 0.00), 5th instar nymphs ($F_{5,474}$ = 617.35, P = 0.00), and total instar nymphs ($F_{5,474}$ = 613.64, P = 0.00). An analysis of the grouping and the similarity degree of nymphs of the common pistachio psylla population was carried out using Duncan's test (Table 2).

Table 1. Means ± standard errors (SEs) of the effect of pesticide + nutrients on the abundance of eggs and the total amount of instar nymphs of *A. pistaciae* in the first year. Mean ± SE is based on the number of egg and nymph density per leaf (see Section 2).

Treatment	Egg	Nymphal periods					
		1	2	3	4	5	Total
A* + N	2.6 ± 0.68b	2.2 ± 0.45b	0.23 ± 0.12b	0.26 ± 0.10a	0.33 ± 0.11b	0.00 ± 0.00a	2.73 ± 0.55c
A + Ca	24.33 ± 14.59a	3.43 ± 0.84ab	0.76 ± 0.24ab	0.53 ± 0.19a	0.60 ± 0.38a	0.13 ± 0.07a	5.46 ± 1.40abc
A + Zn	7.43 ± 3.73ab	5.36 ± 1.29a	0.33 ± 0.13b	0.40 ± 0.11a	0.13 ± 0.06b	0.30 ± 0.18b	6.30 ± 1.41ab
A + N + Ca	2.03 ± 0.46b	3.1 ± 0.75ab	0.66 ± 0.19ab	0.20 ± 0.07a	0.03 ± 0.03b	0.00 ± 0.00b	4.00 ± 0.82abc
A + N + Zn	0.76 ± 0.21b	1.56 ± 0.45b	0.50 ± 0.16ab	0.30 ± 0.15a	0.06 ± 0.04b	0.00 ± 0.00b	2.36 ± 0.59c
A+N + Zn + Ca	2.93 ± 1.2b	1.63 ± 0.35b	0.33 ± 0.13b	0.13 ± 0.06a	0.03 ± 0.03b	0.00 ± 0.00b	2.13 ± 0.43c
A + Zn + Ca	17.4 ± 9.86ab	2.93 ± 0.75ab	0.30 ± 0.12b	0.43 ± 0.18a	0.00 ± 0.00b	0.00 ± 0.00b	3.66 ± 0.94bc
Amitraz	6.86 ± 2.43ab	5.03 ± 1.46a	1.06 ± 0.33a	0.26 ± 0.03a	0.16 ± 0.08b	0.50 ± 0.19a	7.03 ± 1.73a
Control	2.46 ± 0.78b	2.06 ± 0.67b	0.60 ± 0.17ab	0.23 ± 0.12a	0.06 ± 0.04b	0.06 ± 0.04b	3.03 ± 0.87bc

Same letters in a column indicate the lack of significant difference at the 5% level.
*A: amitraz.

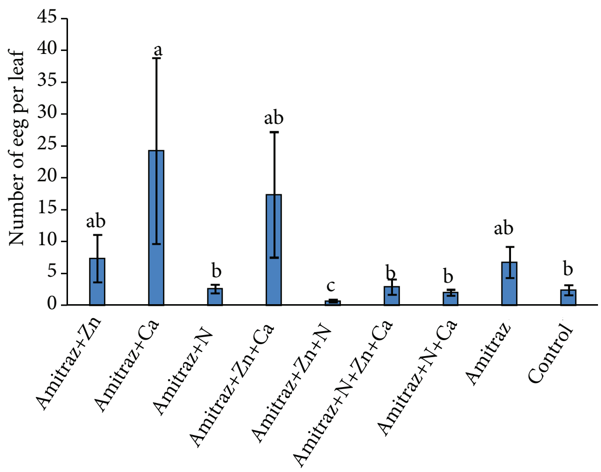

Figure 1. Effect of pesticide + nutrients on the frequency of *A. pistaciae* eggs in the first year.

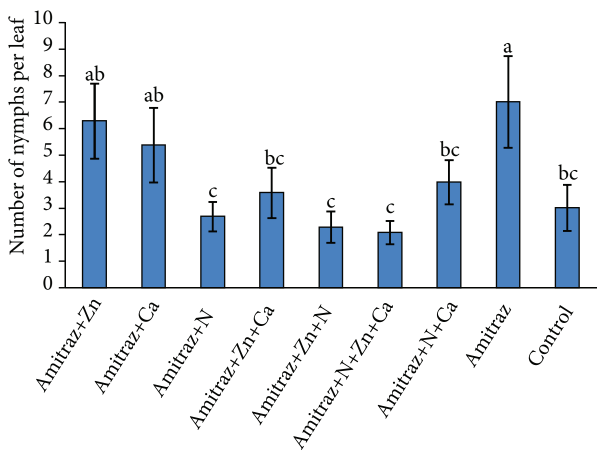

Figure 2. Effect of pesticide + nutrients on the total amount of instar nymphs of *A. pistaciae* in the first year.

The results were similar to those of the first year experiment: abundance of eggs was decreased by amitraz + N + Zn and increased by amitraz + Ca (Figure 3). Results for nymph instars showed that amitraz increased population density significantly, and amitraz + N + Zn + Ca decreased the density of them insignificantly (Figure 4). These results showed that amitraz increased population density of the 1st, 2nd, 3rd, 4th, and 5th nymph instars, and amitraz + N + Zn + Ca decreased population density of them (Table 2).

4. Discussion

Many researchers have studied the effect of nutrient elements, especially nitrogen (Fragoyiannis et al. 2001; Morales et al. 2001; Moon and Stiling 2005; Kagata and Katayama 2006; Lu et al. 2007; Green et al. 2010) and tank-mixing of herbicides with micronutrients (Sander et al. 1987; Tea et al. 2004; Bagherani 2005; Minbashi et al. 2006), but a global literature review showed that no records were available that were related to the mixing of pesticides with micronutrients and the application of the mixture to *A.*

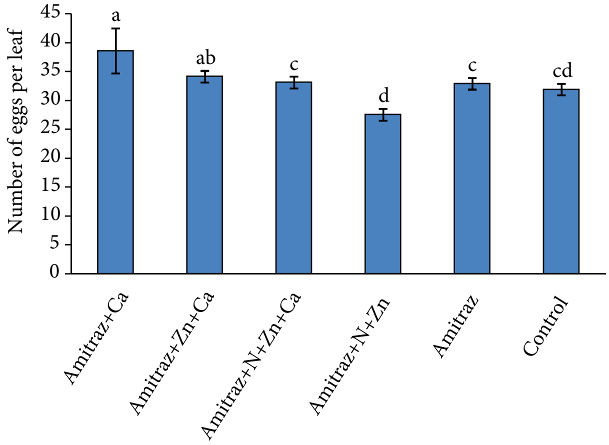

Figure 3. Effect of pesticide + nutrients on frequency of *A. pistaciae* eggs in the second year.

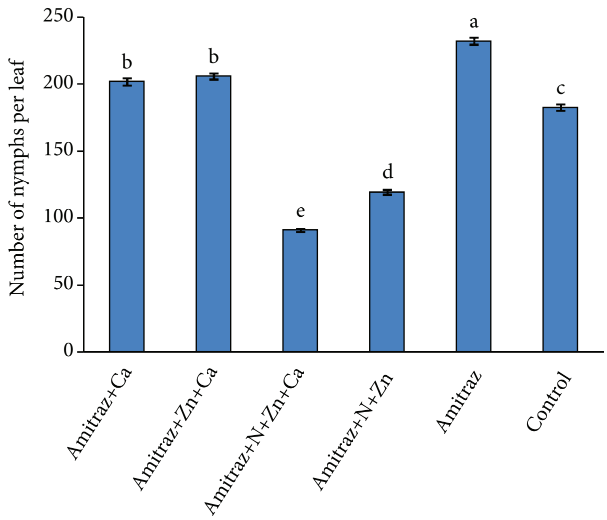

Figure 4. Effect of pesticide + nutrients on the total amount of instar nymphs of *A. pistaciae* in the second year.

Table 2. Mean ± SE of the effect of pesticide + nutrients on the abundance of eggs and the total amount of instar nymphs of *A. pistaciae* in the second year. Mean ± SE is based on the number of egg and nymph density per leaf (see Section 2).

Treatment	Egg	Nymphal periods					
		1	2	3	4	5	Total
A* + Ca	38.63 ± 3.88a	58.05 ± 0.71c	45.87 ± 0.93b	22.52 ± 0.61a	21.65 ± 0.64a	53.97 ± 0.76b	202.08 ± 2.68b
A + N + Zn	34.17 ± 1.01c	39.25 ± 0.76d	26.4 ± 0.56d	15.41 ± 0.44c	14.17 ± 0.37c	24.68 ± 0.72d	119.52 ± 1.81d
A + N + Zn+ Ca	33.15 ± 1.02b	26.57 ± 0.59e	22.25 ± 0.37e	12.38 ± 0.31d	11.11 ± 0.38d	18.68 ± 0.46e	91.01 ± 1.30e
A + Zn + Ca	27.57 ± 0.98ab	61.4 ± 0.73b	46.57 ± 1.03b	23.17 ± 0.61a	22.36 ± 0.40a	53.00 ± 0.87b	206.12 ± 2.32b
Amitraz	32.95 ± 1.01b	70.90 ± 0.52a	54.26 ± 1.18a	22.45 ± 0.52a	21.06 ± 0.57a	63.71 ± 0.80a	232.39 ± 2.59a
Control	31.92 ± 0.99bc	57.01 ± 0.77c	39.73 ± 0.90c	18.53 ± 0.47b	17.01 ± 0.37b	50.57 ± 0.64c	182.88 ± 2.28c

Same letters in a column indicate the lack of significant difference at the 5% level.
*A: amitraz.

pistaciae. When pesticides are used with other pesticides, fertilizers, and additives, synergic (Minbashi et al. 2006) and antagonist (Petroff 2003) interactions occur between the chemicals. Our studies demonstrated that micronutrient tank-mixing with amitraz is possible, which is consistent with other researchers' reports (Sander et al. 1987; Tea et al. 2004; Minbashi et al. 2006), but the mix of amitraz with calcium produces an antagonist phenomenon and decreases the effect of calcium on the abundance of *A. pistaciae*. Tea et al. (2004) indicated that nitrogen with sulfur has potential for successful tank-mixing. Sander et al. (1987) also used tank-mixing of 10 herbicides and 6 liquid fertilizers successfully. Luna (1988) investigated the effects of Ca on *Heliothrips haemorrhoidalis* and *M. persicae* fed on *Phaseolus vulgaris* and Brussels sprouts, respectively, and showed that calcium fertilizer decreased the density of aphids and mites. Samih et al. (2011) reported that calcium decreased the abundance of *A. pistaciae*, which is not in agreement with the results of this study. In this investigation, amitraz increased the density of *A. pistaciae* significantly. Long-term utilization of insecticide causes the appearance of resistance in populations of pests (Metcalf 1982). Long-term application of amitraz, for controlling pistachio psylla, may cause increasing amitraz resistance in this pest. It is clear in our results that the use of the combination of nitrogen, zinc, and calcium reduce the incremental effect of amitraz.

According to these results, it appears that it is possible to mix pesticides with micronutrients without creating chemical or physical incompatibility. This research also suggests that the tank-mix of amitraz with micronutrients is better than the application of amitraz alone because it decreased the incremental effect of amitraz. This is due to the effect of micronutrients on various physiological processes and structures of the plant, as well as the plant vigor, quality, and cell wall structure. Correspondingly, insects can qualitatively distinguish among host plants and can feed preferentially on plants based on quality (Chen et al. 2004; Littke and Zabowski 2007). Calcium affects cell wall structure and physiological processes in trees (Littke and Zabowski 2007), and zinc is essential for the normal healthy growth of plants (Sarwar 2011). Tank-mixing can also save time and labor and may reduce equipment and application costs.

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